

Growth, Production, and Bean Quality of *Coffea arabica* as Affected by Interspecific Grafting: Consequences for Rootstock Breeding

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Abstract. In order to avoid nematode damage to roots of *Coffea arabica* L. in Latin America, a common practice is interspecific grafting on *C. canephora* var. *Robusta* (Pierre) rootstocks. The performance of two *C. arabica* cultivars, 'Caturra' and 'Catimor T5175', was evaluated on four rootstocks: *C. canephora* var. *Robusta* ('T3561' and 'T3757') and *C. liberica* var. *liberica* (Hiern) and var. *dewevrei* (Lebrun), over 5 years in a trial at 1180 m elevation in Costa Rica. Nongrafted plants of the two *Arabica* cultivars were used as controls. Mortality of plants grafted on the two *C. liberica* cvs. was >20% vs. 6% to 13% for plants grafted on *C. canephora*, and 3% to 4% for the two controls. Analysis of accumulated yields over four harvests showed that the rootstocks limited stem girth and reduced yield 10% to 48%. Yield on the *C. canephora* rootstock was greater than that on the two *C. liberica* cultivars. However, grafting did not affect female fertility (peaberries, empty berries) or content of several chemicals, such as caffeine, fat, and sucrose. The two *C. liberica* rootstocks significantly reduced aroma and bean size. Histological studies revealed symptoms of incompatibility, characterized by more dilated and less distinct growth rings and appearance of plugged vascular connections. The poor performance of the rootstocks may therefore be explained by partial incompatibility. However, growth and productivity were also affected by poor adaptations of *C. canephora*, *C. liberica*, and *C. dewevrei* to the lower temperature at high altitudes and by morphological differences in the root systems. These results emphasize the need to develop better adapted rootstock cultivars from *C. canephora* var. *Robusta*.

Grafting of coffee to reduce nematode injury is a well-developed practice in Latin America. In Guatemala, hypocotyledonary grafting has been used on a large scale for >30 years. Villain et al. (1996) observed that with heavy *Pratylenchus* sp. infestations, production of *Arabica* plants grafted on a *C. canephora* var. *Robusta* rootstock was four times as great as that of nongrafted plants. In the presence of heavy *Meloidogyne incognita* (Chitwood) infestations in Brazil, Da Costa et al. (1991)

observed that production of plants grafted on *Robusta* was three to four times greater than that of nongrafted plants. In addition to being used to increase resistance to soilborne parasites, grafting is also commonly used for fruit trees and in silviculture to improve tree adaptation to unfavorable soil or climatic conditions. There is abundant information in the literature on rootstock-scion interactions that affect vigor and productivity. Such results have been reported for apple (*Malus domestica* Borkh.) (Shechter et al., 1991) and peach (*Prunus persica* L.) (Bussi et al., 1996; Glucina et al., 1992; Layne et al., 1976). The rootstock can also influence fruit size (Ogasanovic and Papic, 1995) and leaf nitrogen and amino acid contents (Doroshenko, 1992). Physical or translocated incompatibilities can occur immediately or over time. Translocated incompatibility (Herrero, 1955), also called biochemical incompatibility, is reflected in reduced starch in the rootstock, or accumu-

lation of toxic phenolic compounds in the union.

Interspecific grafting of *Coffea* sp. has been studied for its potential benefits in preserving fragile genetic resources and improving resistance to nematodes. Couturon (1993) reported no incompatibility between *C. arabica* and 10 other *Coffea* species at 600 m elevation: the best species for vigor and low mortality were *C. liberica* and *C. canephora*. In India, Raghuramulu and Thimmaraju (1998) reported excellent compatibility between *C. arabica* and *C. canephora* and *C. liberica* var. *dewevrei*. Partial incompatibility was detected with *C. liberica* var. *liberica*, confirming previous field observations (Cramer, 1957). In Indonesia, Nur (1984) reported differences in growth among *C. arabica* plants grafted on *Robusta* rootstocks from several sources. In a Brazilian study evaluating the efficiency of nematode control via grafting, Melo et al. (1976) detected no difference in caffeine content between *Arabica* grafted on *Robusta* and nongrafted controls.

No studies are known in which the influence of rootstock on coffee bean quality has been evaluated. The objectives of this study were to: 1) evaluate the effect of interspecific grafting on yield and bean quality of *Arabica* coffee produced in an area with optimum growing conditions; and 2) obtain information on how to improve the selection of rootstock cultivars.

Materials and Methods

Plant material. The plant material used in this study is described in Table 1. Grafting was carried out with two *Coffea arabica* cultivars ($2n = 4x = 44$) used as scions, and rootstocks from two diploid ($2n = 2x = 22$) species of *Coffea* using Reyna's hypocotyledonary grafting technique (1966). The *Arabica* cultivars were 'Caturra', widely produced in Latin America, and 'Catimor T5175', a new cultivar resistant to leaf rust (*Hemileia vastatrix* Berk. et Br.) that yields 20% to 25% more than does 'Caturra'. The rootstocks were from open-pollinated progenies of the following diploid species: *C. canephora* var. *Robusta* (Froehner); *C. liberica* var. *liberica* (Hierns); and *C. liberica* var. *dewevrei* (Lebrun) Lebrun. In this study, the latter two species are referred to as *C. liberica* and *C. dewevrei*, respectively. Note that these two subspecies reach a height of >6–8 m and a stem girth between 80 and 130 cm under free growth conditions, whereas *C. canephora* reaches a height of 4–5 m and 40–75 cm in girth, and *C. arabica* cultivars 2–3 m in height and 20–30 cm in girth. Following grafting, plants were grown in plastic bags under greenhouse conditions for 7 months before being planted in the field.

Experimental design. The trial was set up in June 1992, at the Coffee Research Centre (CICAFE), Heredia, Costa Rica, at 1180 m elevation, with an annual average temperature of 20.5 °C, on an Andosol soil, and with 2200 mm of annual rainfall. The experimental design consisted of seven randomized treatments, with single-tree plots, and 25–35 replicates

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Table 1. Origins of scions and rootstocks.

Scion	Origin	Source	Rootstock	Origin	Source	No. trees studied
T5175 (Catimor)	CIFC HW 26/13	ICAFE	Nongrafted (control)	---	ICAFE	35
T5175 (Catimor)	CIFC HW 26/13	ICAFE	Robusta, T3561 (2-1)	Rep. of Congo, L48	CATIE	25
Caturra	Brazil	ICAFE	Nongrafted (control)	---	ICAFE	35
Caturra	Brazil	ICAFE	Robusta, T3757 (1-2)	Indonesia SA-13	CATIE	30
Caturra	Brazil	ICAFE	Robusta, T3561 (2-1)	Rep. of Congo, L48	CATIE	33
Caturra	Brazil	ICAFE	Liberica, T3718 (1-1)	Africa	CATIE	33
Caturra	Brazil	ICAFE	Dewevrei, T3446 (1-1)	Puerto Rico	CATIE	29

(trees). Plant spacing was 2 m between rows and 1 m within rows. The trial was conducted without shade. Plants received 1000 kg·ha⁻¹ of 18N–3P–10K–8Mg–0.5B fertilizer annually, split into two applications in May and August, 250 kg·ha⁻¹ of N in November, and two foliar applications per year of copper hydroxide (1.5 kg·ha⁻¹) to prevent leaf and fruit diseases.

Growth. Stem girth (10 cm above ground level) was measured 17 and 66 months after planting, and total height of the stem and average length of the two longest branches after 66 months. After the fourth harvest, the trees were cut back to 20 cm above ground level. New shoots were counted five months later and classified according to their growth status, using a scale of 1–5, where 1 = poor development and 5 = good development. Ability to produce shoots after stumping was estimated by an index of stumping response (shoot number × shoot quality). Six months after stumping, when the trees had resprouted well, samples of 20 leaves were collected from a total of 10 trees from each grafting combination and the control. Content of N, K, Mg, Ca, Al, Zn, and Cu was analyzed. Nitrogen was determined by the Kjeldahl method (Jones and Case, 1990) and other elements by atomic absorption spectrometry. Phosphorus was quantified by spectrometry at 660 nm, following a colorimetric method with molybdenum blue. Data are expressed in percentage of dry matter.

At the end of the experiment, all the plants were carefully removed to study their root systems. Number and length of taproots and abundance of secondary roots were evaluated on a scale of 1–5, where 1 = very few roots and 5 = abundant roots. For 10 plants from each graft combination and the control, the stem was cut crosswise for macroscopic observation of the graft union. A histological technique was used to observe the graft unions of two 'Caturra' trees grafted on: 1) Robusta 'T3561'; 2) Robusta 'T3757'; 3) *C. liberica*; and one control tree. All grafted trees observed were growing poorly. The graft union was cut into segments 2 cm long and a microtome was used to cut 25-μm slices from the segments. The samples were fixed for 24 h in a solution of FAA and then dehydrated in an ethanol series (50–70–80–90–95–100–100%) for 30 min each. Safranin (2%) was added in 70% ethanol and fast green (1%) in 100% ethanol for 15 s. The slices were placed in a solution of 1 absolute ethanol : 1 xylene for 30 s and then in pure xylene (2× for 30 min each) (Sass, 1958), before being examined under the microscope.

Fertility and yield. Cumulative yields (grams of fresh berries per tree) of surviving trees over 4 years were recorded. Coffee berries normally contain two seeds, but abortion of one ovule during early fruit development gives rise to one round seed, called peaberry (Van der Vossen, 1985). Another postzygotic abortion can occur when the fruit has attained its final size. This late abortion gives rise to one or two empty locules, causing the berry to float when immersed in water (De Reffye, 1975). The berries from the last three harvests were scored for: 1) fraction of peaberries in a subsample of 100 green fruits collected 6 months after flowering from the eight most heavily bearing nodes; 2) fraction of floating berries in a subsample of 200 ripe fruits after immersion in water; and 3) fraction of beans in a subsample of 2 kg of green coffee retained by a size 17 sieve (6.65 mm round holes).

Chemical analyses and cup quality tests. The effects of grafting on chemical composition and organoleptic properties were studied for 3 consecutive years (the second, third, and fourth harvest years). Samples of 250 g of green coffee were collected after screening through a size 17 sieve and eliminating most defective beans. Sucrose, chlorogenic acid, caffeine, trigoneline, and fat contents were obtained by chemical analysis after grinding the beans, following the method of Guyot et al. (1988). The analysis were achieved by near infrared spectrometry by reflectance of green coffee after grinding (ground to <0.5 mm). An NIR spectrometer system (model 6500; NIRSystem, Silver Spring, Md.) driven by NIRS2 (4.0) software (Intrasoft Intl. LLC, Port Matilda, Pa.). Each compound was evaluated using specific calibrations. Cup quality tests were carried out after controlled roasting (weight loss and color measurement) on an infusion prepared from 50 g of roasted coffee. A panel of eight persons tasted, under white light environment, 50–60-mL samples kept at 50–60 °C. The main taste and beverage attributes (aroma, body, acidity, bitterness, and astringency) were estimated using scales ranging from 0–5, where 0 = nil, 1 = very light, 2 = light, 3 = regular, 4 = strong, and 5 = very strong. An additional preference score was used ranging from 0–5, where 0 = not good for drinking, 1 = very bad, 2 = bad, 3 = regular, 4 = good, and 5 = very good.

Statistical analysis. All data were subjected to a one-way analysis of variance (ANOVA) using SAS version 6.04 (SAS Institute, 1988). For categorical data, the normality of residuals and homoscedasticity were verified to about fulfill the conditions for

ANOVA. Duncan's multiple range test was used for mean separation. To improve comparisons, the 'Catimor T5175' control was compared with the 'Catimor T5175' grafted on Robusta 'T3561', while the 'Caturra' control was compared with other treatments. The differences were considered significant at $P \leq 0.05$.

Results and Discussion

Effects on mortality and growth. Mortality was higher for plants grafted on *C. liberica*, or *C. dewevrei* (Table 2) than for controls, whereas plants grafted on Robusta tended to show similar or slightly higher mortality than did the controls. 'Caturra' grafted on Robusta 'T3561' grew significantly better than did the other grafted plants; after 1 year in the field, the height and stem girth were not significantly different from that of nongrafted plants (Table 2). After 66 months of growth, the effect of grafting on stem girth remained unchanged. On average, plants grafted on *C. canephora* were taller whereas those grafted on *C. liberica* and *C. dewevrei* were shorter than the nongrafted controls. Plants with the strongest branch growth were the controls and 'Caturra' grafted on Robusta 'T3561', which again differed significantly from the remaining grafted plants.

Rootstocks also had a significant effect on quality and the number of resprouting shoots after stumping (Table 3). The 'Caturra' scions grafted on *C. dewevrei* had a significantly lower response index than did all other plants. As for chemical contents of the leaves, only the aluminum content differed significantly; aluminum content in leaves of trees on rootstocks that imparted low vigor was 23% higher. The root system was significantly affected by grafting in 'Caturra' (Table 3), nongrafted plants had a root system without a developed main taproot, but with several short pseudo-taproots and dense secondary roots. In contrast, the *C. dewevrei* and the *C. liberica* rootstocks had one or two very large taproots with a low density of secondary roots. The Robusta rootstocks were intermediate. The roots of 'T3561' resembled those of 'Caturra', while the roots of 'T3757' behaved like *C. dewevrei* roots. The root systems of *C. dewevrei* and *C. liberica* and, to a lesser extent, of Robusta 'T3757', differed from that of 'Caturra'. The presence of a deep taproot associated with a low density of fine roots, affects soil exploration and consequently nutrient uptake capacity. This may explain the much higher leaf aluminum contents for the

Table 2. Effects of rootstock on mortality, growth, and production of two scion cultivars of coffee.

Scion/rootstock	Mortality (%)	Trunk girth (cm)		Height (cm)	Branch length (cm)	Fruit yield (g/tree)	Coefficient of variation (%) ^z
		17 mo	66 mo				
T5175 (control)	2.9	7.5 a ^y	18.2 a	180 a	97.8 a	17593 a	32.7
T5175/T3561 (Robusta)	13.3	5.8 b	16.5 b	187 a	87.7 b	13718 b	48.8
Caturra (control)	4.0	7.3 a	18.1 a	183 ab	95.6 a	15772 a	38.7
Caturra/T3561 (Robusta)	6.0	6.9 a	17.1 ab	198 a	94.0 ab	14106 ab	41.1
Caturra/T3757 (Robusta)	13.3	5.7 ab	16.4 b	185 a	83.7 a–c	9786 b	61.7
Caturra/ <i>C. liberica</i>	21.2	5.2 b	16.4 b	164 c	88.6 bc	9305 b	67.5
Caturra/ <i>C. dewevrei</i>	20.7	5.0 b	16.8 b	171 bc	86.6 c	8130 b	66.9

^zThe coefficient of variation is based on the four cumulative yields.^yMean separation within columns and scion cultivars by Duncan's multiple range test, $P \leq 0.05$.

Table 3. Effects on rootstock on growth after stumping, abundance of secondary roots, length of taproots, leaf aluminum content, and berry characteristics, of two scion cultivars of coffee.

Scion/rootstock	Growth after stumping ^z	Abundance ^y of secondary roots	Length of tap roots (cm)	Aluminum (mg·kg ⁻¹)	Peaberries ^x (%)	Floating berries ^w (%)	Heavy beans ^v (%)
T5175 (control)	14.5 a ^u	3.8 a	61 a	109.8 a	8.4 a ^y	7.6 a	49.3 a
T5175/T3561 (Robusta)	10.5 a	3.0 a	58 a	101.0 a	8.6 a	5.4 a	45.8 a
Caturra (control)	11.0 a	3.2 a	56 b	101.5 b	10.0 a	4.0 a	40.5 a
Caturra/T3561 (Robusta)	14.5 a	2.8 ab	48 b	101.0 b	9.6 a	2.8 a	36.7 a
Caturra/T3757 (Robusta)	12.5 a	2.1 b	108 a	123.0 a	10.2 a	3.2 a	39.5 a
Caturra/ <i>C. liberica</i>	11.5 a	1.8 b	116 a	133.0 a	11.8 a	3.8 a	31.2 b
Caturra/ <i>C. dewevrei</i>	8.5 b	2.0 b	112 a	134.0 a	11.4 a	3.0 a	33.6 ab

^zShoot numbers × shoot quality. Shoot quality was scored according to a scale ranging from 1–5, where 1 = very poor growth and 5 = very good growth.^yScored according to a scale ranging from 1–5, where 1 = very few and 5 = abundant roots.^xFraction of peaberries in a subsample of 100 green fruits collected 6 months after flowering.^wFraction of floating berries in a subsample of 200 ripe fruits after immersion in water.^vFraction of heavy beans retained by a no. 17 screen.^uMean separation within columns and scion cultivars by Duncan's multiple range test, $P \leq 0.05$.

three rootstocks with deep taproots. In this type of soil, aluminum content in fact increases in the lower horizons.

Effects on yield. The rootstocks induced variable and lower yields in comparison with those of the controls (Table 2). The Robusta 'T3561' rootstock reduced accumulated yield 10% and 22% in 'Caturra' and 'Catimor T5175', respectively. This effect was greater for the Robusta 'T3757' rootstock, with a 38% reduction. *Coffea liberica* and *C. dewevrei* rootstocks reduced yield 41% to 48%, respectively, relative to 'Caturra'. Coefficients of variation were higher for the less productive combinations. Classification of treatments remained the same irrespective of the numbers of replicates considered (all plants or only the 10 best plants of each treatment). The higher production and vigor of nongrafted *C. arabica* cultivars with those grafted on the *C. liberica* or *C. dewevrei* and Robusta rootstocks was quite noticeable. These characteristics revealed

themselves early on, resulting in lower mortality and a more rapid increase in stem height and girth. This difference was maintained in stem vigor and production for the whole productive cycle, although inter- and intraspecific differences in behavior were recorded. *Coffea canephora* (var. Robusta) was apparently more adapted to local conditions than were *C. liberica* and *C. dewevrei*. Within the Robusta species, the 'T3561', originating from Republic of Congo, was more adapted than was the 'T3757' from Indonesia. Yield of the latter was well below that of the controls. The 23% difference in yield between Robusta 'T3561' and Robusta 'T3757' can be explained mainly by the morphological differences in their root systems.

Effects on fertility and cup quality. The scions had significant effects on the number of peaberries and empty berries, whereas the rootstock did not significantly affect these two characteristics (Table 3). The scion also had a

stronger influence on bean size than did the rootstock. Larger beans were produced on 'T5175' than on 'Caturra'. There was no effect of grafting on Robusta. In contrast, plants grafted on *C. liberica* and *C. dewevrei* tended to produce smaller beans (Table 3). Grafting had no effect on chlorogenic acid, trigonelline, caffeine and fat contents, nor did organoleptic tests reveal differences between grafted and nongrafted plants (Table 4), except for aroma, for which graft combinations with *C. liberica* and *C. dewevrei* were inferior to the control. The species used as rootstocks in the present study produce coffee of inferior quality, with a biochemical composition different from that of *C. arabica*; for example, the caffeine content ranges between 1.4% to 4% for Robusta and 1.0% to 1.8% for *C. liberica* vs. 0.8% to 1.2% for Arabica (Louarn, 1992). However, our study demonstrated that interspecific grafting did not affect biochemical composition or cup quality, although it affected bean size and

Table 4. Effects of rootstock on cup quality of coffee from two scion cultivars (average for 3 years).

Scion/rootstock	Aroma ^z	Body	Acidity	Bitterness	Astringency	Preference score
T5175 (control)	2.93 a ^y	2.75 a	1.90 a	1.80 a	2.30 a	3.00 a
T5175/T3561 (Robusta)	2.93 a	2.70 a	1.75 a	2.85 a	2.15 a	2.75 a
Caturra (control)	2.90 ab	2.85 a	2.15 a	2.70 a	2.30 a	3.10 a
Caturra/T3561 (Robusta)	3.20 a	3.15 a	2.00 a	3.25 a	2.45 a	3.10 a
Caturra/T3757 (Robusta)	3.40 a	2.55 a	1.75 a	2.85 a	2.00 a	3.50 a
Caturra/ <i>C. liberica</i>	2.53 b	2.65 a	1.75 a	2.80 a	2.10 a	2.80 a
Caturra/ <i>C. dewevrei</i>	2.70 b	2.60 a	1.75 a	2.85 a	2.20 a	2.75 a

^zScores for aroma, body, acidity, bitterness, and astringency estimated using scales ranging from 0–5, where 0 = nil and 5 = very strong. Preference score, 0–5, where 0 = nil and 5 = very good. Data obtained from a panel of eight judges.^yMean separation within columns and scion cultivars by Duncan's multiple range test, $P \leq 0.05$.

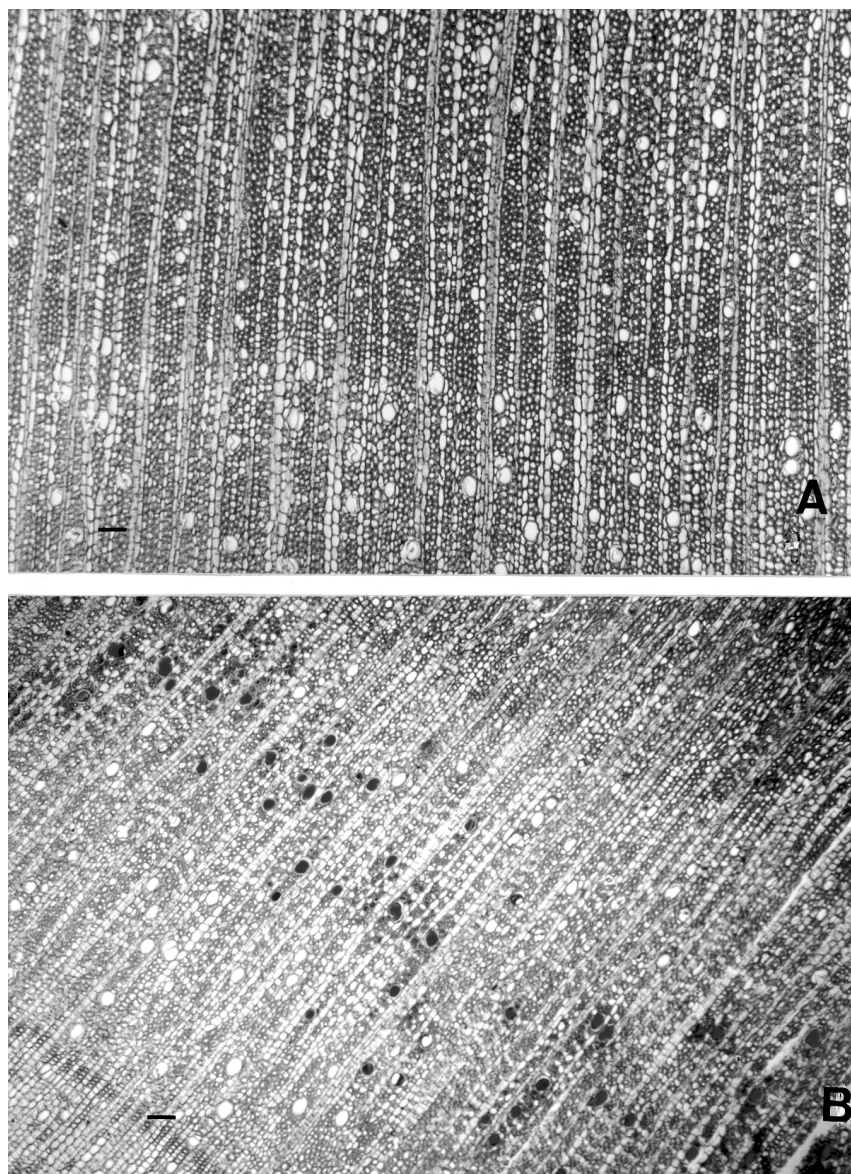


Fig. 1. Xylem vessels in (A) nongrafted 'Caturra' trunk and in (B) cross-section of graft union of 'Caturra' x *C. liberica* coffee. The black patches are plugged xylem vessels. Bar = 300 μ m.

aroma. The factors that most affect bean size are water supply (Suárez-Serrato, 1978) and bean : leaf ratio (Cannell, 1974). Poor vegetative growth on *C. liberica* and *C. dewevrei* explains their effects in reducing bean size.

Scion/rootstock compatibility. More than half the plants grafted on *C. dewevrei* or *C. liberica* had thickened graft unions. This was probably the result of differences in growth rate, hence in diameter, between rootstocks and scions rather than incompatibility. Macroscopic evaluation of longitudinal stem sections did not reveal any abnormalities at the graft union and there was no sign of necrosis. This confirms that there is no physical incompatibility in the combinations studied. Such results have been reported in early stages by Raghuramulu and Thimmaraju (1998) and by Couturon (1993). However, for the six trees chosen for poor growth, histological evaluation revealed differences between control and

grafted trees. Sections of graft unions from Robusta rootstocks ('T3757' and 'T3561') differed slightly from the controls. They were more dilated with less distinct growth rings, and sometimes appeared to have plugged vascular connections. The same phenomena, but more pronounced, were observed in *C. liberica* (Fig. 1). These plants had symptoms of translocated incompatibility characterized by plugged vascular connections, probably the result of an accumulation of toxic phenolic compounds. The sample of plants used in the histological study was too small to show whether this phenomenon was involved in all of the *C. canephora* and *C. liberica* plants. Nevertheless, this hypothesis is probable since the grafted plants always yielded less than did the nongrafted plants, even when considering only the best ten plants in each combination. The occurrence of such incompatibility is probably temperature-related. Both *C. canephora*

and *C. liberica* grow best in the humid tropics areas with average temperatures of 25 °C or more. Reported exceptions are a *C. canephora* population well adapted to 1300 m elevation in Sudan and one *C. liberica* population on Mont Tonkoui at 1000 m in the Ivory Coast. Most indigenous populations are located between 300 and 600 m. At lower temperatures, *C. canephora* tends to exhibit symptoms characteristic of cold lesions, with poor plant growth and high mortality. For example, the average height of 100 plants from two *C. canephora* clones growing in a plot next to the trial described in this study (elevation 1180 m) was 113 cm after 25 months. The same plants cultivated in a plot at 600 m reached a height of 174 cm. Moreover, in low-lying areas, Arabica plants are more vigorous when grafted on Robusta than on their own roots (Couturon, 1993).

Rootstock breeding program. The morphology of the root system should become an important criterion when selecting new rootstock cultivars. The strong correlation observed between stem girth at 17 months and yield ($r^2 = 0.76$) (data not shown) also shows that early selection of rootstocks is possible. For further rootstock breeding programs, the simultaneous use of four criteria is recommended: 1) juvenile mortality; 2) stem girth at 15–20 months; 3) morphology of the root system; and 4) resistance to soilborne parasites.

Given these results, interspecific grafting at optimum altitude for Arabica (elevation 1000–1300 m), with average temperatures between 20 to 23 °C, is recommended only in the presence of highly pathogenic nematodes. This is a very common situation in Guatemala and El Salvador for extensive areas located at elevations >1000 m. In the case of infestation with less pathogenic species (i.e., *M. exigua* Goeldi, Bertrand et al., 1998), resistance of the rootstock may not compensate for the loss of productivity. Grafting on Arabusta (*C. arabica* x *C. canephora*) interspecific hybrid rootstocks ($2n = 2x = 44$) is worth considering as an alternative. In Africa, the high vigor of Arabusta has been recorded at elevations ranging from 400 to 1000 m. A program has been initiated in Central America to develop an Arabusta rootstock. Following in vitro multiplication through somatic embryogenesis of two parents selected for their resistance to *Meloidogyne* sp., a Robusta tetraploid produced by colchicine application and a male-sterile Ethiopian accession, field plots will be established to produce seeds of a new Arabusta cultivar. At low altitudes, grafting on Robusta or other *Coffea* sp. should be studied for its potential in adapting Arabica coffee to marginal conditions. In Central America, the Nemaya cultivar, an excellent rootstock for controlling soilborne parasites (Bertrand et al., 2000), is now available to producers.

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